STUDIES OF THE DISTRIBUTION OF IMMUNITY TO YELLOW FEVER IN BRAZIL.

II. THE DISPROPORTION BETWEEN IMMUNITY DISTRIBUTION AS REVEALED BY COMPLEMENT-FIXATION AND MOUSE-PROTECTION TESTS AND HISTORY OF YELLOW FEVER ATTACK AT CAMBUCY, RIO DE JANEIRO.*

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Introduction.

The postepidemic distribution of immunity to yellow fever in the population of Magé, Rio de Janeiro, as measured by the complement-fixation and *Macacus rhesus*-protection tests (1), indicated that the number of persons acquiring immunity to this disease during an outbreak may greatly exceed the number of clinical cases observed. Recently, the mouse-protection test (2) has largely supplanted the monkey-protection test and has made possible more extensive studies of immunity distribution than were formerly feasible. As a control for the wide-spread studies of immunity distribution which are being undertaken throughout Brazil, and which are based on relatively small samples from a large number of towns, it was deemed necessary to make an intensive survey of some single community where the history of yellow fever was known. The town of Cambucy, Rio de Janeiro, was chosen for this purpose. Fortunately the history of yellow fever in this community is similar to that in Magé, so data for the two towns can be compared. The results of epidemiological and immunity distribution studies in Cambucy, which are reported in the following pages, confirm the observations made at Magé that reintroduction of the yellow fever virus into native populations of previously

* The work on which this report is based was undertaken as a part of a larger field study of the epidemiology of yellow fever in Brazil being made by the Cooperative Yellow Fever Service maintained by the Brazilian Government and The Rockefeller Foundation. The laboratory tests were divided between the New York and Bahia yellow fever laboratories, both supported by The Rockefeller Foundation.

endemic areas may produce wide-spread immunity with few typical cases of the disease. But such wide-spread immunity may be far from uniform in its distribution within even small population groups.

Description of Cambucy.

Cambucy, a small town with a population of less than one thousand, is situated on the Parahyba river, in the northern part of the State of Rio de Janeiro, about 80 kilometers from Campos, a city of almost fifty thousand population, and one of the most important sugar producing centers of Brazil. Cambucy lies outside the heavy sugar producing zone and depends for its existence largely upon the production of coffee in the hilly region north of the Parahyba river. It is only 50 meters above sea-level and has a warm, humid climate (table 1). The

TABLE 1.

Meteorological data for Campos.

1930

Months 1930	Temperature (Degrees Centigrade)		Relative humidity	Rainfall (mm.)
	Average of maxima	Average of minima		
January	33.3	21.8	75.9	95.3
February	31.9	21.7	80.0	107.5
March	31.2	21.3	81.5	114.4
April	28.3	19.9	85.2	204.4
May	27.3	18.3	83.0	40.0
June	28.8	17.2	82.0	48.4
July	26.8	16.3	81.5	34.6
August	26.9	17.1	79.1	18.5
September	26.2	17.0	80.0	35.0
October	27.6	19.3	82.3	112.0
November	27.2	18.9	82.2	200.0
December	29.1	21.0	85.2	281.5

Leopoldina Railway connects Cambucy with both Rio de Janeiro and Nictheroy, which may be reached via Campos in only eleven hours, and via Tres Irmãos and Portella in thirteen hours.

The census taken as a part of the present study revealed a population of 823, of which 42, or 5 per cent, were foreign born. Ninety-one persons were born in Brazil of foreign parents, and forty-four others reported having one foreign parent. From these figures it appears that the population of the town is about 85 per cent native stock. Despite this fact, however, the racial distribution found was: white,

500; black, 135; mulatto, 187; Indian, 1. Females predominated over males in the ratio of 110 to 100. These data and those for age distribution will be found summarized in table 6.

History of yellow fever in Cambucy.

No written record of the occurrence of yellow fever in Cambucy prior to the epidemic here described has been found, but it is believed that the disease must have been present in the town repeatedly during the long period of endemicity in the Federal District and the State of Rio de Janeiro, from 1849 to 1908. How long it may have persisted in this area after the final success of the memorable campaign of Oswaldo Cruz in Rio de Janeiro is unknown, but no autochthonous cases were reported from the entire state during two full decades from 1908 to 1928. The last case of yellow fever reported from Campos during this early endemic period occurred in 1906.

In May, 1928, yellow fever was found in the Federal Capital, and in the following months of 1928 it appeared in the nearby points of Nictheroy and São Gonçalo. During 1929 it was present in the Federal District until July, and was reported from many places * in the State of Rio de Janeiro. The entire northern part of the state, in which Campos and Cambucy are situated, failed to report locally infected cases during 1929. From September, 1929, until late in March, 1930, no cases were reported from the State of Rio de Janeiro, but between the end of March, 1930, and July of the same year, five towns reported the disease. Four of these, Cantagallo, Itaocára, Portella, and Campos, lie in the northern part of the state and are in relatively close contact with Cambucy. This flare-up was followed by four months of respite, during which detachments of non-immune revolutionary troops from Minas Geraes were moved through this region, apparently without becoming infected. During the last week of November, however, a fatal case, confirmed by autopsy, occurred in Cambucy, and a total of thirteen clinical cases, with five deaths, was registered before the middle of February, 1931. Other suspected cases, with at least one death, occurred in the nearby rural districts.

Description of control points, Friburgo and Guapé.

As a control for the results of mouse-protection tests in Cambucy, two towns were selected in which the transmission of yellow fever had never been reported, namely Friburgo, in the State of Rio de Janeiro, and Guapé in the State of Minas Geraes.

* Including Magé, the scene of the preceding study.

Friburgo lies about 100 kilometers north of the city of Rio de Janeiro, on the branch line of the Leopoldina Railway, which connects Portella with the federal and state capitals. It is well within the previously endemic area, but has apparently always been protected from yellow fever epidemics by its location at 850 meters above sea-level. A typical clinical case of yellow fever was observed in Friburgo in 1931; but it did not give rise to any registered locally infected cases, and a mosquito survey at that time failed to reveal the presence of *Aëdes aegypti.*

Guapé is situated in the southern part of the State of Minas Geraes, at an altitude of more than 600 meters above sea-level, and has no easy contact with known endemic areas. Although occasional epidemics of an undiagnosed fever of short duration have been reported from this town, it is not known to have suffered from yellow fever.

Collection of data and material.

Early in February, while yellow fever was still present in Cambucy, a complete census was taken of the population of the community, including, in addition to the usual census data, information as to place of birth and history of recent illness. Blood specimens were secured during February and March from 659 of the 823 persons listed in the census. Unsatisfactory results were obtained in a large proportion of the mouse-protection tests made on these specimens, owing to the technical difficulties encountered in the early days of this test. For this reason and because of a desire to measure the rate of loss of complement-fixing bodies, a second collection of blood specimens was made in November and December of the same year, in which 598 specimens were obtained.

The first series of blood specimens collected during and immediately after the epidemic was divided. One portion was forwarded to the yellow fever laboratory in New York for the mouse-protection test, and the other portion was sent to the yellow fever laboratory in Bahia, Brazil, for the complement-fixation test. The second series of specimens went to the Bahia laboratory, where both mouse-protection and complement-fixation tests were performed.

In September, 1931, 105 blood specimens were collected in Friburgo from persons between the ages of two and twenty years who had resided in the community continually since birth. At the same time, 136 specimens were collected at Guapé, largely from persons of the earlier age groups, although some were obtained from members of the older groups who had never resided outside this area. All sera from

Friburgo and Guapé were forwarded to the Bahia laboratory for the mouse-protection test.

Laboratory and statistical methods.

The complement-fixation and mouse-protection tests were carried out by the workers who originally described these tests. The complement-fixation test was performed with antigens prepared from the livers of monkeys infected with yellow fever (4). No recent modifications have been made in this test. The procedure in the mouse-protection test consisted of the intraperitoneal injection of virus fixed for mice and of the serum being tested for protective substances, and the intracerebral injection of an inert irritant (2). Six animals were used for each serum tested, and each run was carefully controlled for virulence of virus used. The few monkey-protection tests reported were carried out in accordance with the method previously described (1).

The only statistical formulae used in the present article are those for the probable error of a percentage, for the probable error of the difference of two percentages, and for chi-square as a measure of association.

(1) $$\text{P.E.}_{\text{Percentage}} = 0.67449\sqrt{\frac{pq}{n}},$$

Where p = percentage positive
q = percentage negative
n = total number on which percentages are based.*

(2) $$\text{P.E.}_{\text{Difference of percentage}} = \sqrt{(\text{P.E.}_{\text{1st percentage}})^2 + (\text{P.E.}_{\text{2nd percentage}})^2}$$

(3) $X^2 = \Sigma\frac{x^2}{m}$, where m is the number expected and $m + x$ the number observed in the four classes of a contingency table (3).

In the statistical discussion a difference four or more times as large as its probable error has been accepted as probably significant. Chi-square results have not been interpreted in probabilities, since such values have been calculated only from four-fold contingency tables, in which n, or the degree of freedom, equals one. Any value of chi-square greater than 6.635 appearing in this report, then, indicates a probability of such distribution occurring by chance less than once in 100 trials. Such probability diminishes rapidly for higher values of chi-square.

* For n less than 15, (1) becomes $0.67449\sqrt{\frac{pq}{(n-2)}}$.

Results of preliminary control.

Seventy-nine sera of persons under twenty years of age, who had lived since birth at Friburgo, Rio de Janeiro, and 135 sera of persons of various ages, always resident in Guapé, Minas Geraes, were examined by the mouse-protection test at the Bahia Laboratory. All sera from Friburgo, and 133 sera from Guapé, gave negative results; two specimens from Guapé which had given positive (5/6) * results were found negative on reexamination.

Additional survey data are now available from various points in Brazil, with percentages of positive-mouse-protection tests ranging from zero to 93. These results agree with those since reported by Hughes and Sawyer (5) for tests of Chinese, Canadian and American sera, and indicate that when the intraperitoneal protection test is performed with mice of the strain now being used, falsely positive results will rarely be recorded. Likewise, falsely positive complement-fixation results have been shown to be infrequent in tests of sera from known non-infectible areas. Soper, et al. (1), reported only three apparently falsely positive reactions in 101 tests on sera from Piracicaba, São Paulo.

There is some indication that the mouse-protection test is more sensitive than the monkey-protection test. Mouse-protection tests were performed on fifteen sera which were still available from the Magé study (1). Of these, thirteen (86.7 $\pm$ 6.4 per cent) were positive, whereas of seventy-six sera examined by the monkey-protection test during that study only forty-two (55.3 $\pm$ 3.8) per cent were found positive. Although the number examined is small, the difference in these percentages (31.4 $\pm$ 7.5) is more than four times its probable error and is in all likelihood significant. Only one of the thirteen sera found positive by the mouse-protection test had been previously examined by the monkey-protection test, and a negative result had been recorded.

Presentation of Cambucy data.

Information indicating recent prevalence of yellow fever in Cambucy. The two practicing physicians living in Cambucy were unable to indicate any definitely suspected clinical cases of yellow fever occurring in the town prior to the fatal case diagnosed in the last week of November, 1930. Both stated their belief, however, that cases of so-called grippe, without respiratory symtoms, roughly estimated as 250 in number, which were observed as early as October and continued

* (5/6) indicates that five of the six mice injected survived.

to the time of the present study, might well have been mild yellow fever infections. Dr. Lannes, who is immune to yellow fever, observed several of these cases in his own home, but was not himself attacked.* Dr. Dantas, who, as physician for the Leopoldina Railway Company, travelled much to nearby towns, stated that similar cases had been seen in other towns along the line for several months before the diagnosis of the first Cambucy case. He agreed that yellow fever had probably been present in Cambucy itself at least two months before a typical case was recognized.

Unfortunately, the Cambucy mortality registration district includes a large rural section, and deaths occurring in the town are not listed separately. A study of mortality records for the Cambucy district (tables 2 and 3) failed to reveal any sudden increase in deaths in

TABLE 2.

Registered deaths in the Cambucy Registration District from 1920 to 1931, by months.

Month	1920	1921	1922	1923	1924	1925	1926	1927	1928	1929	Average deaths 1920–29	1930	1931
January...	11	7	8	12	8	13	12	13	9	19	11.2	15	☐
February..	11	18	8	5	9	21	18	9	10	16	12.5	7	☐
March....	15	5	11	3	6	19	12	5	7	9	9.2	11	8
April.....	9	8	12	12	7	11	12	9	14	7	10.1	9	6
May.....	9	13	8	8	12	11	19	13	6	15	11.4	6	6
June.....	4	6	10	7	9	16	9	14	10	17	10.2	9	9
July.....	6	11	12	5	16	18	12	11	11	9	11.1	11	5
August....	6	13	14	11	10	14	6	13	8	9	10.4	9	9
September.	6	4	15	3	11	9	6	8	6	7	7.5	12	14
October...	7	11	5	6	16	17	13	6	4	13	9.8	7	15
November.	4	7	13	11	15	10	5	4	13	15	9.7	☐	13
December .	10	10	7	14	16	26	9	6	10	20	12.8	☐	11
Annual....	98	113	123	97	135	185	133	111	108	156	125.9	109	109

☐ Months in which yellow fever is known to have been present in Cambucy.

1930 which could be attributed to undiagnosed yellow fever. On the contrary, the mortality recorded during the known epidemic period, November, 1930, to February, 1931, was lower than for any correspond-

* Of nineteen persons residing in the Lannes home, six who were over twenty years of age reported no history of recent illness, although two reported having had yellow fever in 1895. Five of this group were tested, and all showed immunity. Of thirteen persons less than twenty years of age, eight gave a history of recent illness, but none definitely suggestive of yellow fever at any time. Nine of this group were tested and seven proved to be immune.

ing period during the previous ten years. Unexpectedly large numbers of burials occurred in Cambucy in 1925 and 1929. In 1925 yellow fever was not known to exist in South Brazil, whereas, in 1929 the disease

TABLE 3.

Registered deaths in the Cambucy Registration District from 1920 to 1930, by age groups.

Year	Age group							Total
	Under 1 year	1–4 years	5–9 years	10–14 years	15–19 years	20–49 years	50 and above	
1920	18	25	13	5	6	17	14	98
1921	30	20	7	3	6	23	24	113
1922	21	21	9	5	3	31	33	123
1923	26	20	5	3	0	18	25	97
1924	42	27	10	4	1	19	32	135
1925	54	48	8	3	7	34	31	185
1926	46	22	7	5	2	30	21	133
1927	34	16	4	3	4	20	30	111
1928	35	26	4	2	3	14	24	108
1929	54	27	8	5	7	27	28	156
Total	360	252	75	38	39	233	262	1259
Average	36.0	25.2	7.5	3.8	3.9	23.3	26.2	125.9
1930	38	20	3	2	0	25	21	109

was widespread in the State of Rio de Janeiro. No explanation was offered for the increased burials of 1929, but the excess during 1925 is attributed by local authorities to the heavy floods of that year which prevented burials in the adjoining districts of São João de Paraizo, Vallão de Padre Antonio, and Alto de Tres Barras. The low number of burials during the epidemic period may be due in part to the unwillingness of families living outside the town to bring their dead to a known fever center.

In the course of the census taken in February, 1931, in Cambucy, in connection with the present study, each family was asked to give information regarding illnesses which had occurred among its members during the previous twelve months. Such data must admittedly have many defects, owing to the faulty memories of untrained observers, many of whom have only approximate ideas of the passage of time. However, it is believed that the data secured are relatively accurate for the six-month period preceding the taking of the history. In analyzing

these histories, it was found that only an occasional illness was reported prior to the preceding October. This was taken to indicate that no exceptional increase of sickness had occurred during the early months of 1930. Information on illnesses reported as occurring after the first of October is summarized by time of occurrence in table 4 and chart 1,

TABLE 4.

Occurrence of recent illness in Cambucy by fortnights and months, October, 1930, to March, 1931.

Fortnight	History of recent illness		
	Number of cases	Month	Number of cases
		1930	
First	5	October	12
Second	7		
Third	5	November	14
Fourth	9		
Fifth	26	December	63
Sixth	37		
		1931	
Seventh	50	January	98
Eighth	48		
Ninth	34	February	37
Tenth	3		
Eleventh	3	March	3
Twelfth	0		
Total	227	Total	227

and by symptoms in table 5. It is apparent that, between October, 1930, and March, 1931, a rather wide-spread epidemic of a relatively mild type, characterized chiefly by fever, headache, and body pains, and, to a less extent, by nausea, vomiting, and icterus, was present in Cambucy. This epidemic gathered headway slowly in October and November, spread rapidly in December, reached its peak in January, and declined rapidly in February. During the period covered by this epidemic, a total of thirteen typical cases of yellow fever, with five fatalities, was reported.

The distribution by houses of reported cases of illness, of clinical cases of yellow fever, and of deaths attributed to yellow fever was

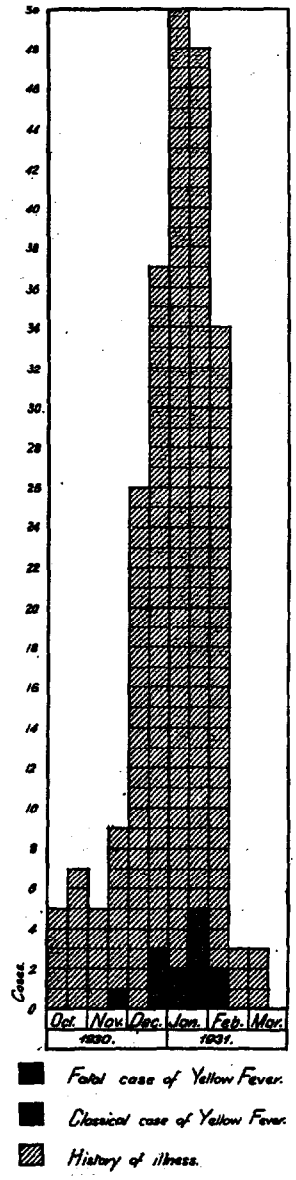

CHART 1. Reported illness by 15-day periods from October 1930 to March 1931. —Cambucy.

TABLE 5.

Distribution of declared symptoms and signs among individuals reporting recent illness, by age groups.

Age group	Census	History of illness Oct., 1930 Mar., 1931	Percentage of group with history of illness	Fever	Headache	Body pains	In bed	Nausea	Vomiting	Icterus	Epigastric pain	Conjunctival congestion	Bleeding gums	Albuminuria
0–4	89	17	19.1 ± 2.8	17	16	15	15	11	13	2	1	0	0	0
5–9	105	33	31.4 ± 3.1	32	29	27	28	13	14	3	4	0	1	0
10–14	94	26	27.7 ± 3.1	25	25	23	24	8	8	1	2	0	0	0
15–19	121	41	33.9 ± 2.9	40	40	40	34	11	11	4	3	2	0	1
20–29	157	49	31.2 ± 2.5	48	49	48	48	20	18	6	5	2	0	1
30–39	98	25	25.5 ± 3.0	25	24	23	20	11	8	0	2	1	0	0
40–49	79	20	25.3 ± 3.3	20	20	20	17	10	8	6	4	0	1	0
50–59	45	11	24.4 ± 4.3	11	11	11	11	2	3	0	0	0	0	0
60–69	17	4	23.5 ± 6.9	4	4	4	4	1	0	0	0	0	0	0
70+	18	1	5.6 ± 3.7	1	1	1	1	0	0	0	0	0	0	0
Total	823	227	27.6 ± 1.0	223	219	212	202	87	83	22	21	5	2	2
Percentage of persons ill reporting a given symptom				98.2 ±0.6	96.5 ±0.8	93.4 ±1.1	89.0 ±1.4	38.3 ±2.2	36.6 ±2.2	9.7 ±1.3	9.3 ±1.3	2.2 ±0.7	0.9 ±0.4	0.9 ±0.4

TABLE 4.

Summary of results of laboratory tests on sera from Cambury.

Age in years. Color, sex	Census	First complement-fixation test*			Second complement-fixation test†			Final complement-fixation test‡			Mouse-protection test		
		Number examined	Number positive	Per cent positive	Number examined	Number positive	Per cent positive	Number examined	Number positive	Per cent positive	Number examined	Number positive	Per cent positive
0–4	89	1	0	.0 —	3	0	.0 —	3	0	.0 —	4	1	25.0±20.7
5–9	105	27	4	14.8±4.6	54	7	13.0±3.1	65	11	16.9±3.1	70	36	51.4± 4.0
10–14	94	61	14	23.0±3.6	68	8	11.8±2.6	77	18	23.4±3.3	75	39	52.0± 3.9
15–19	121	81	16	19.8±3.0	87	15	17.2±2.7	107	28	26.2±2.9	92	48	52.2± 3.5
20–29	157	86	22	25.6±3.2	113	14	12.4±2.1	131	31	23.7±2.5	116	70	60.3± 3.1
30–39	98	61	12	19.7±3.4	69	9	13.0±2.7	81	18	22.2±3.1	74	43	58.1± 3.9
40–49	79	42	6	14.3±3.6	57	6	10.5±2.7	64	12	18.8±3.3	63	48	76.2± 3.6
50–59	45	25	3	12.0±4.4	32	1	3.1±2.1	36	4	11.1±3.5	32	21	65.6± 5.7
60–69	17	9	1	11.1±8.0	14	4	28.6±8.8	15	4	26.7±7.7	14	11	78.6± 8.0
70+	18	10	2	20.0±9.5	14	1	7.1±5.0	16	3	18.8±6.6	15	12	80.0± 7.0
Total	823	403	80	19.9±1.3	511	65	12.7±1.0	595	129	21.7±1.1	555	329	59.3± 1.4
Under 20	409	170	34	20.0±2.1	212	30	14.2±1.6	252	57	22.6±1.8	241	124	51.5± 2.2
Over 20	414	233	46	19.7±1.8	299	35	11.7±1.3	343	72	21.0±1.5	314	205	65.3± 1.8
Under 40	664	317	68	21.5±1.6	394	53	13.5±1.2	464	109	23.5±1.3	431	237	55.0± 1.6
Over 40	159	86	12	14.0±2.5	117	12	10.3±1.9	131	20	15.3±2.1	124	92	74.2± 2.6
White	500	240	49	20.4±1.8	308	40	13.0±1.3	359	79	22.0±1.5	336	201	59.8± 1.8
Black	135	60	10	16.7±3.2	84	10	11.9±2.4	98	19	19.4±2.7	91	58	63.7± 3.4
Mulatto	187	103	21	20.4±2.7	119	15	12.6±2.1	138	31	22.5±2.4	128	70	54.7± 3.0
Indian	1	0	0	.0 .0	0	0	.0 .0	0	0	.0 .0	0	0	.0 .0
Men	392	186	33	17.7±1.9	250	17	6.8±1.1	285	46	16.1±1.5	263	146	55.5± 2.1
Women	431	217	47	21.7±1.9	261	48	18.4±1.6	310	83	26.8±1.7	292	183	62.7± 1.9

* Specimens secured at end of epidemic, February and March, 1931.

† Specimens secured eight months later, November and December, 1931.

‡ Combined results counting all cases with a positive reading on either test as positive.

carefully studied. The first fatal case recorded from No. 28 Rua Treze de Maio, on November 27, was preceded in October by undiagnosed illness in Nos. 28 and 40 in the same block. There is a very close association between cases of the relatively mild illness reported by the population of Cambucy and recognized cases of yellow fever, although they do not definitely establish such relationship. Additional grounds for the belief that this epidemic was neither dengue nor any other mild infection, but really yellow fever, will be found in the results of laboratory tests submitted in this report.

Distribution of immunity to yellow fever in Cambucy, as shown by complement-fixation and mouse-protection tests. The results of complement-fixation and mouse-protection tests on sera collected in Cambucy are summarized in table 6. It will be seen that the results of the complement-fixation tests are remarkably uniform throughout, with the exception of those for the second tests in men, which are unexpectedly low, and those for the second tests in women, which are unexpectedly high. The difference in percentage (11.4 ± 1.9) is almost six times its probable error.

TABLE 7.

Comparative results of first and second complement-fixation tests on persons tested twice with an interval of eight months between tests.

First complement-fixation test	Second complement-fixation test		
	Positive	Negative	Total
Positive	16	47	63
Negative	18	238	256
Total	34	285	319

Value $\chi^2 = 18.0$

Percentage positive, first test ..... 19.7 ± 1.5

Percentage positive, second test ..... 10.7 ± 1.2

Difference in percentage positive ..... 9.0 ± 1.9

$D/PE = 4.7$

The percentage of positive complement-fixation results on specimens drawn eight months after the epidemic (12.7 ± 1.0) is significantly lower than the percentage of positive results on specimens drawn in February and March (19.9 ± 1.3). When comparison is made of the results of first and second complement-fixation tests on the sera of the same person (table 7), this reduction in percentage

positive for the whole group is unaltered (19.7 ± 1.5 to 10.7 ± 1.2). The statistical analysis of table 7 indicates clearly that the results of first and second complement-fixation tests are closely associated. However, only one-fourth (25.4 ± 3.9) of the specimens positive on the first test were positive on the second and a small percentage (7.4 ± 1.1) of those negative on the first test were positive on the second.

The shift from positive to negative is statistically significant and indicates that a rapid loss of complement-fixing bodies, similar to that reported for Magé (1), occurred in Cambucy convalescents during the months following the epidemic. The shift from negative to positive may have been due to the occurrence of actual infections after the first specimens were taken, but it was more probably due to an increase of complement-fixing bodies in cases first bled shortly after infection.

A study of the results of the first complement-fixation tests according to the interval elapsing between the reported illness and the collection of the blood sample (table 8) indicates that a significantly higher percentage of positive results are to be expected when the sample is taken from six to ten weeks after attack than when it is taken at an earlier or a later time. In this connection it may be mentioned that Frobisher (6) estimated that complement-fixing bodies reach a maximum concentration in the blood of *M. rhesus* after a lapse of thirty to forty days.

The percentage of positive mouse-protection tests on specimens drawn eight months after the epidemic, was lower than the percentage of positive results on specimens drawn in February and March. One hundred and nineteen positive tests were read in a total of 170 first examinations (70.0 ± 2.37 per cent) performed in New York, as compared with 311 positives in 522 second examinations (59.6 ± 1.43 per cent) performed in Bahia. This apparent reduction (10.4 ± 2.78 per cent) is almost four times its probable error, but very likely does not signify an actual loss of protecting properties with the passage of time, since the results of the first mouse-protection tests, analyzed according to the period elapsing between the suspected attack and the date of the drawing of blood, failed to show any significant difference such as was observed in the results of the complement-fixation test. Furthermore, successive examinations of sera from the same persons failed to show a reduction in the percentage of positives found.*

* Sera of 137 persons were examined in both series. Of these, 102 were positive on first examination and 99 were positive on second examination. Five persons with negative results in the first examination were found positive eight months later. These may possibly represent immunities developed between the taking of the first and the second specimens, since the virus is known to have been present in Cambucy

TABLE 8.

Results of first complement-fixation test and first mouse-protection test, according to interval elapsing between suspicious illness and drawing of blood sample.

Interval between illness and drawing of blood sample	First complement-fixation test						First mouse-protection test			
	Number examined	Number positive	Number examined	Number positive	Per cent positive	Difference in per cent positive	Number examined	Number positive	Per cent positive	Difference in per cent positive
One week	7	3								
Two weeks	4	1								
Three weeks	6	2	32	8	25.0 ± 5.2		24	20	83.3 ± 5.1	
Four weeks	9	0								
Five weeks	6	2				30.6 ± 7.2				No
Six weeks	10	5				*D/PE* = 4.25				significant
Seven weeks	9	4								difference
Eight weeks	9	8	45	25	55.6 ± 5.0		24	21	87.5 ± 4.6	
Nine weeks	11	5								
Ten weeks	6	3								
Eleven weeks	4	0				36.8 ± 8.3				No
Twelve weeks	8	2				*D/PE* = 4.44				significant
Thirteen weeks	0	0								difference
Fourteen weeks	1	0								
Fifteen weeks	0	0	16	3	18.8 ± 6.6		15	13	86.7 ± 5.9	
Sixteen weeks	2	1								
Seventeen weeks	0	0								
Eighteen weeks	1	0								
Nineteen weeks	0	0								
	93	36	93	36	38.7 ± 3.4		63	54	85.7 ± 3.0	

TABLE 9.

Results of complement-fixation and mouse-protection tests on persons with and without history of recent illness.

History of recent illness	First complement-fixation test				Second complement-fixation test				Final complement-fixation results				Mouse-protection test			
	Positive	Negative	Total	Per cent positive	Positive	Negative	Total	Per cent positive	Positive	Negative	Total	Per cent positive	Positive	Negative	Total	Per cent positive
Positive	36	57	93	38.7 ± 3.4	31	118	149	20.8 ± 2.3	56	106	162	34.6 ± 2.5	145	17	162	89.5 ± 1.6
Negative	44	266	310	14.2 ± 1.3	34	328	362	9.4 ± 1.0	73	360	433	16.9 ± 1.2	184	209	393	46.8 ± 1.7
Total	80	323	403	19.9 ± 1.3	65	446	511	12.7 ± 1.0	129	466	595	21.7 ± 1.1	329	226	555	59.3 ± 1.4

Values for χ^2.

History of illness with first complement-fixation 26.9
History of illness with second complement-fixation 12.3
History of illness with final complement-fixation 21.8
History of illness with mouse-protection test 86.2

Difference in per cent positives in persons with and without history of illness:

First complement-fixation ..... 24.5 ± 3.6 *D/PE* 6.8
Second complement-fixation ..... 11.4 ± 2.5 *D/PE* 4.6
Final complement-fixation ..... 17.7 ± 2.8 *D/PE* 6.3
Mouse-protection test ..... 42.7 ± 2.3 *D/PE* 18.6

TABLE 10.

Mouse-protection and final complement-fixation results classified by age groups and by history of recent illness.

History of recent illness	Mouse-protection test								Final complement-fixation results							
	19 years and under				20 years and over				19 years and under				20 years and over			
	Positive	Negative	Total	Per cent positive	Positive	Negative	Total	Per cent positive	Positive	Negative	Total	Per cent positive	Positive	Negative	Total	Per cent positive
Positive.....	73	5	78	93.6 ± 1.9	72	12	84	85.7 ± 2.6	27	46	73	37.0 ± 3.8	29	60	89	32.6 ± 3.4
Negative....	51	112	163	31.3 ± 2.4	133	97	230	57.8 ± 2.2	30	149	179	16.8 ± 1.9	43	211	254	16.9 ± 1.6
Total.....	124	117	241	51.5 ± 2.2	205	109	314	65.3 ± 1.8	57	195	252	22.6 ± 1.8	72	271	343	21.0 ± 1.5

Values for χ^2

History of recent illness with mouse-protection
- 19 years and under 82.3
- 20 years and over 21.2

History of recent illness with final complement-fixation
- 19 years and under 12.2
- 20 years and over 9.7

Difference in per cent mouse-protection positives in groups with and without history of recent illness

19 years and under	62.3 ± 3.1	*D/PE*	20.1
20 years and over	27.9 ± 3.4	*D/PE*	8.2

Difference in per cent mouse-protection positives in groups 19 years and under and 20 years and over

with history of recent illness	7.9 ± 3.2	*D/PE*	2.5
without history of recent illness	26.5 ± 3.3	*D/PE*	8.0

Difference in per cent complement-fixation positives in groups with and without history of recent illness

19 years and under	20.2 ± 4.2	*D/PE*	4.8
20 years and over	15.7 ± 3.8	*D/PE*	4.1

Difference in per cent complement-fixation positives in groups 19 years and under and 20 years and over

with history of recent illness	4.4 ± 5.1	*D/PE*	0.9
without history of recent illness	0.1 ± 2.5	*D/PE*	0.04

Relationship between history of illness and results of complement-fixation and mouse-protection tests. A comparison of the distribution of positive laboratory tests in the two groups, persons with history of recent illness and persons without such history (table 9), revealed a high degree of association between positive history of recent illness and positive laboratory results.* This association was more marked with the mouse-protection test than with the complement-fixation tests. The first complement-fixation test showed a higher degree of association with history of recent illness than did the second one, although even in the latter a high degree of association was found.

When the two groups, classified as to final complement-fixation results and results of mouse-protection tests, are further divided into the age groups, 19 years and under, and 20 years and over (table 10), a much higher degree of association is found between the results of the mouse-protection test and history of recent illness in the group 19 years and under than in the group 20 years and over. No such difference is found in the relation of complement-fixation results and history of recent illness for these two age groups. A further analysis of history of illness and complement-fixation and mouse-protection tests by groups above and below certain ages (table 11 and chart 2) showed that there were definite tendencies toward increase in positive reactions to mouse-protection tests in persons over twenty years of age and toward a decrease in histories of illness in persons over forty years of age, but no demonstrable tendency toward variation with age in results of the complement-fixation test.

The results of complement-fixation tests on the groups with and without history of recent illness were compared. The group with history of recent illness dropped from 38.7 $\pm$ 3.4 per cent positive on first examination to 20.8 $\pm$ 2.3 per cent positive on second examination. The difference (17.9 $\pm$ 4.1 per cent) is 4.4 times its probable error, and is believed to be significant. The group without history of

when this study began. On the other hand, eight specimens which were found positive in New York and were later found negative in Bahia, probably do not represent any loss of immunity. It is possible that these apparently false positives were due to the presence of resistant animals among the mice used in the early days of the mouse-protection test. In the present study these thirteen cases were included with the results of the second examination made at Bahia, with the exception of one case which was clearly positive when examined in New York and (2/6) negative when examined in Bahia.

* Of twenty-eight persons who volunteered the information that they had been sufficiently ill to call a physician during the period covered by the present study, twenty-six (92.9 $\pm$ 3.3 per cent) were found by the mouse-protection test to be immune to yellow fever.

recent illness showed only a slight reduction from 14.2 ± 1.3 per cent to 9.4 ± 1.0 per cent, a difference 4.8 ± 1.6 per cent, on the second test.

TABLE 11.

History of recent illness, and results of complement-fixation and mouse-protection tests below and above given ages.

Age in years	Per cent positive below given age			Per cent positive above given age		
	History of illness	Complement-fixation	Mouse-protection	History of illness	Complement-fixation	Mouse-protection
10	25.8 ± 2.1	16.2 ± 3.0	50.0 ± 3.9	28.1 ± 1.2	22.4 ± 1.2	60.7 ± 1.5
20	28.6 ± 1.5	22.6 ± 1.8	51.5 ± 2.2	26.6 ± 1.5	21.0 ± 1.5	65.3 ± 1.8
30	29.3 ± 1.3	23.0 ± 1.4	54.3 ± 1.8	23.7 ± 1.8	19.3 ± 1.8	68.2 ± 2.2
40	28.8 ± 1.2	22.8 ± 1.3	55.0 ± 1.6	22.6 ± 2.2	17.6 ± 2.2	74.2 ± 2.6
50	28.4 ± 1.1	22.3 ± 1.2	57.7 ± 1.5	20.0 ± 3.0	16.4 ± 3.1	72.1 ± 3.9
60	28.2 ± 1.1	21.6 ± 1.2	58.2 ± 1.4	14.3 ± 4.0	22.6 ± 5.1	79.3 ± 5.1
70	28.1 ± 1.1	21.8 ± 1.2	58.7 ± 1.4	5.6 ± 3.7	18.8 ± 6.6	80.0 ± 7.0

The original data on which calculations of table 11 are based may be found in tables 5 and 6.

For final comparison of the results of complement-fixation tests with results of other tests, a list of final complement-fixation results was made, in which all persons with positive results on either first or second test were considered positive. On this basis, a highly significant difference (17.7 ± 2.8 per cent) was found between the percentage of positives (34.6 ± 2.5) in the group with history of recent illness and that (16.9 ± 1.2) in the group without history of recent illness. The complement-fixation results, on the other hand, showed no great tendency to accompany in variation the values of history of illness and mouse-protection tests.

Although Cambucy is very irregularly divided into blocks, an attempt was made to study the history of illness and results of laboratory tests by blocks (table 12). Very much higher history of illness rates and correspondingly higher mouse-protection rates were found in the more densely populated blocks. Although the probable errors of these rates were large because of the small numbers examined in each block, the relationship of one series to the other was quite evident (chart 3). The relationship of the results of complement-fixation tests to those and the other two series was not so evident. When the town was roughly divided into a thickly populated and a sparsely populated area highly significant corresponding differences were found in the history of recent illness and the results of mouse-protection tests, but no signifi-

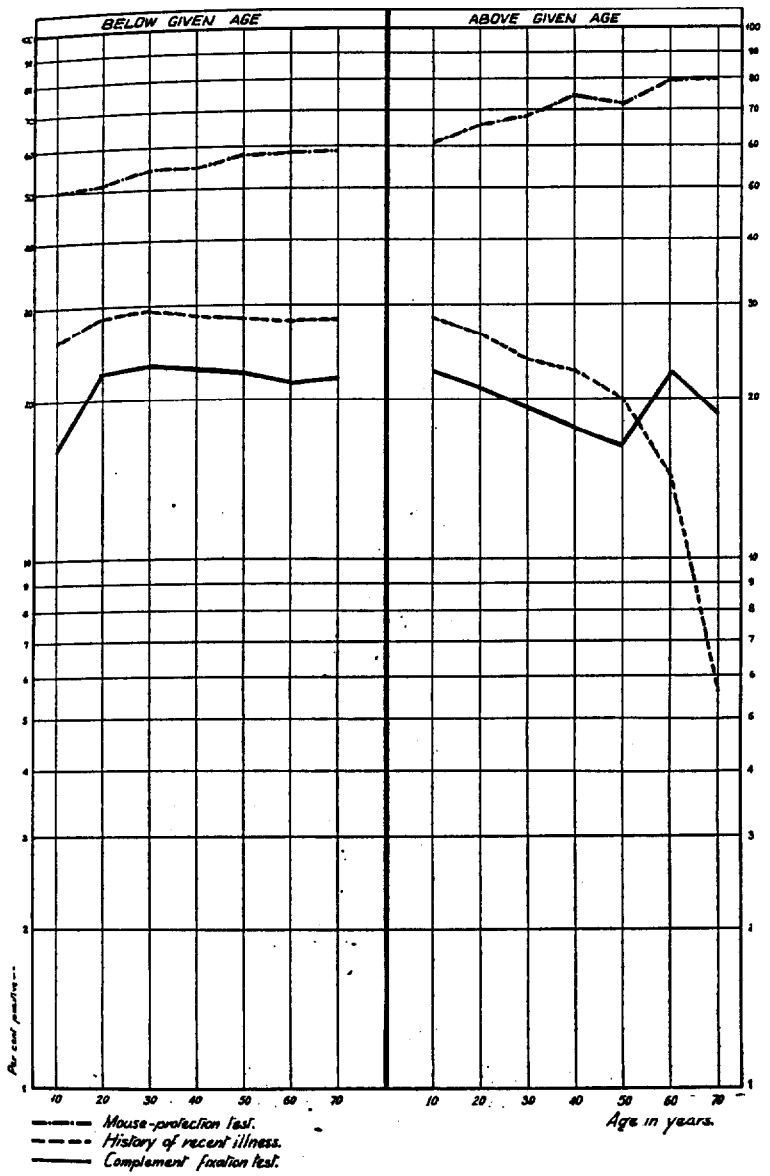

CHART 2. Results of mouse-protection, complement-fixation and history of recent illness. Investigations in population groups above and below given age.

TABLE 12.

Distribution of history of illness, and results of complement-fixation and mouse-protection tests by blocks, Cambucy.

	History of recent illness				Final complement-fixation result				Results of mouse-protection test			
Block number	Positive	Negative	Total	Per cent positive	Positive	Negative	Total	Per cent positive	Positive	Negative	Total	Per cent positive
1	48	66	114	*42.1* ± 3.1	17	69	86	*19.8* ± 2.9	74	10	84	*88.1* ± 2.4
2	46	70	116	39.7 ± 3.1	13	63	76	17.1 ± 2.9	61	16	77	79.2 ± 3.1
3	35	44	79	44.3 ± 3.8	19	43	62	30.6 ± 3.9	36	16	52	69.2 ± 4.3
4	14	21	35	40.0 ± 5.6	5	22	27	18.5 ± 5.0	18	9	27	66.7 ± 6.1
5	5	17	22	22.7 ± 6.0	6	10	16	37.5 ± 8.2	10	5	15	66.7 ± 8.2
6	5	9	14	35.7 ± 9.3	5	8	13	38.5 ± 9.9	7	5	12	58.3 ± 10.5
7	19	38	57	33.3 ± 4.2	8	33	41	19.5 ± 4.2	22	16	38	57.9 ± 5.4
8	31	85	116	26.7 ± 2.8	16	69	85	18.8 ± 2.9	40	35	75	53.3 ± 3.9
9	9	73	82	11.0 ± 2.3	11	40	51	21.6 ± 3.9	24	24	48	50.0 ± 4.9
10	4	37	41	9.8 ± 3.1	6	25	31	19.4 ± 4.8	10	12	22	45.5 ± 7.2
11	3	26	29	10.3 ± 3.8	3	16	19	15.8 ± 5.6	9	13	22	40.9 ± 7.1
12	5	49	54	9.3 ± 2.7	10	32	42	23.8 ± 4.4	10	29	39	25.6 ± 4.7
13	2	14	16	12.5 ± 5.6	3	9	12	25.0 ± 9.2	3	9	12	25.0 ± 9.2
14	1	18	19	5.3 ± 3.5	3	9	12	25.0 ± 9.2	2	7	9	22.2 ± 10.6
15	0	16	16	0.0 —	3	8	11	27.3 ± 10.0	2	11	13	15.4 ± 7.3
16	0	13	13	0.0 —	1	10	11	9.1 ± 6.5	1	9	10	10.0 ± 7.2
Town	227	596	823	27.6 ± 1.0	129	466	595	21.7 ± 1.1	329	226	555	59.3 ± 1.4
Zone I	200	382	582	34.4 ± 1.3	89	335	424	21.0 ± 1.3	272	123	395	68.9 ± 1.6
Zone II	27	214	241	11.2 ± 1.4	40	131	171	23.4 ± 2.2	57	103	160	35.6 ± 2.6
Area A + B	106	121	227	46.7 ± 2.2	36	126	162	22.2 ± 2.2	142	15	157	90.4 ± 1.6
Remainder	121	475	596	20.3 ± 1.1	93	340	433	21.5 ± 1.3	187	211	398	47.0 ± 1.7

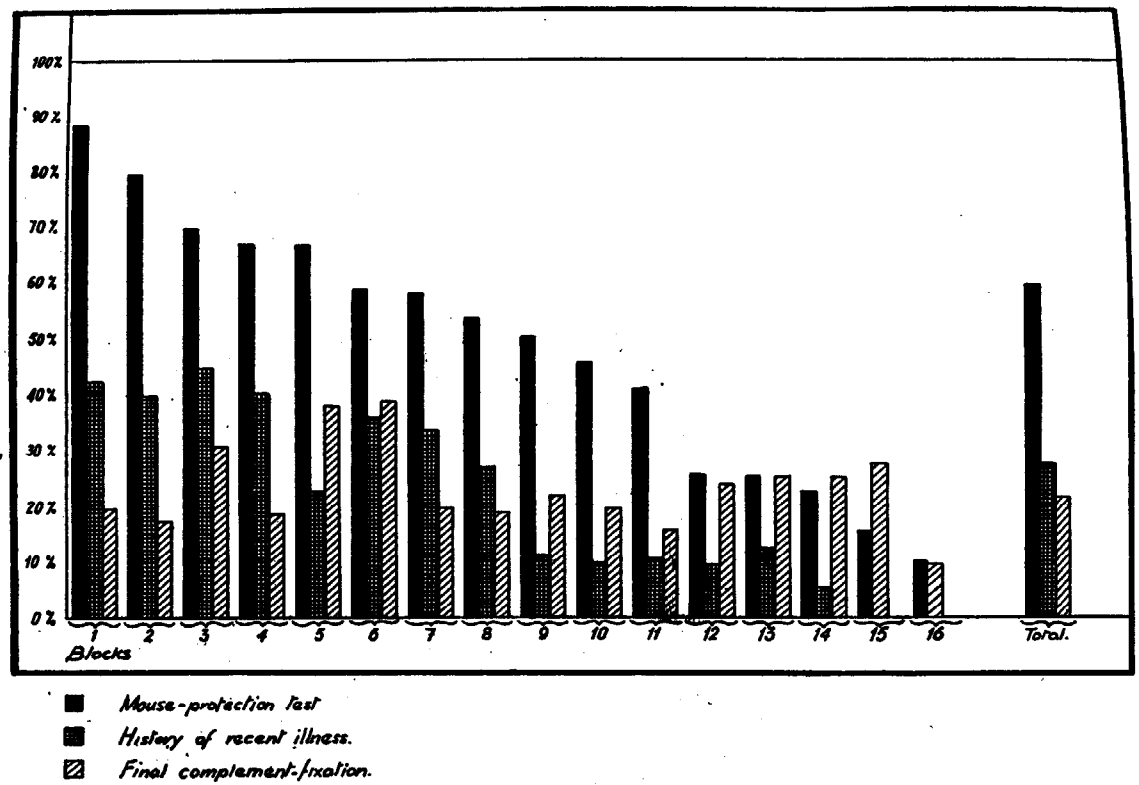

CHART 3. Distribution of history of illness, complement-fixation and mouse-protection, results by blocks.—Cambucy.

cant difference was noted in results of complement-fixation tests. Similar results are found in comparing special areas A and B with the rest of the town.

Despite the differences noted in the results of complement-fixation and mouse-protection tests, these tests are far from being independent of each other, as is clearly shown by the significant differences in percentages of positive reactions in the various groups of table 13, and the

TABLE 13.

Comparative results of complement-fixation and mouse-protection tests.

Results of complement-fixation test			Results of mouse-protection test				Difference in per cent positive	Difference / Probable error
First	Second	Final	Positive	Negative	Total	Per cent positive		
Positive	Positive		13	1	14	92.9 ± 5.0		
Negative	Negative		94	120	214	43.9 ± 2.3	49.0 ± 5.5	8.90
Positive	Negative		35	8	43	81.4 ± 4.0		
Negative	Positive		10	6	16	62.5 ± 8.2	18.9 ± 9.12	2.07
Positive			54	12	66	81.8 ± 3.2		
Negative			120	145	265	45.3 ± 2.1	36.5 ± 3.82	9.55
	Positive		49	11	60	81.7 ± 3.4		
	Negative		228	176	404	56.4 ± 1.7	25.3 ± 3.80	6.65
		Positive	90	22	112	80.4 ± 2.5		
		Negative	208	188	396	52.5 ± 1.7	27.9 ± 3.02	9.23

Value of χ^2

Final complement-fixation with mouse-protection..... 27.9

high value of chi-square for association of final complement-fixation results with mouse-protection results. As was previously reported for monkey-protection and complement-fixation tests (1), a much higher percentage (81.7 ± 2.3) of positive than of negative tests (48.0 ± 1.3) was confirmed by mouse-protection results. The percentages of first and second positive complement-fixation tests confirmed by mouse-protection results were the same, whereas a significantly lower percentage of negatives was confirmed for the second test (43.6 ± 1.7) than for the first (54.7 ± 2.1). This is a further indication that there is a loss of complement-fixing bodies with the passage of time.

Birth outside of epidemic areas did not seem to greatly affect history of recent attack or the results of laboratory tests. Of fifty-five

persons born in regions not subject to yellow fever, twenty-three (41.8 ± 4.48) gave a history of recent illness. This was possibly significantly higher than the rate of attack for the entire population (27.6 ± 1.0) ($D/PE = 3.12$), although the twenty-three positive reactions (59.0 ± 5.3) obtained in the examination of thirty-nine persons in this group do not suggest a higher rate of immunity for the group than is enjoyed by the community as a whole (59.3 ± 1.4 per cent). Practically the same percentage was found (fourteen positives in twenty-five examinations, or 56.0 ± 6.7 per cent) for persons who had resided less than twenty years in Cambucy as for the entire group born in non-endemic areas. The mouse-protection rates for this group, classified as to history of attack, were 77.8 ± 6.6 per cent for those with history of recent illness, and 42.9 ± 6.3 per cent for those without such history. These rates were not significantly different from similar rates for the entire group, which were 89.6 ± 1.6 per cent and 45.8 ± 1.7 per cent respectively.

Discussion.

In considering the information on laboratory tests presented above, and their relationship to declared history of recent illness, the intrinsic differences and limitations of complement-fixation and protection tests (1) should be remembered. The complement-fixing bodies are apparently less constantly produced and are certainly less permanent in character than are the protection bodies. Complement-fixing bodies appear more tardily than do protection bodies, and apparently increase over a period of some weeks, only to decline rapidly. This does not mean, however, that all positive complement-fixation tests can be attributed to recent illness, since Frobisher (6) and Davis (7) have shown independently that reexposure to the virus is sufficient to produce an increase in the concentration of complement-fixing bodies in the blood of monkeys previously immunized, whose complement-fixing titer had fallen very low. The finding of any considerable percentage of positive complement-fixation results in a given population, then, should be interpreted as probably indicating that this community has recently harbored the virus of yellow fever, but does not necessarily imply a recently acquired immunity to the disease in every case. On the other hand, the finding of a high percentage of animal-protection tests gives no indication of the time of immunization, except in sera from the younger age groups. Although the protection bodies are apparently constantly produced and permanent in character, the test for their presence is a biological one, and, as such, subject to occasional

variation. The mouse-protection test has, then, an important advantage over the monkey-protection test, in that the result is not based on a single animal, but on several. The comparative results of monkey-protection and other tests are shown for a small series of persons in table 14. The monkey-protection results are lower than were anticipated on the basis of the other results for the same group. All positive monkey tests were confirmed by mouse tests, but the mouse test disclosed five positives apparently missed by the monkey test.

TABLE 14.

Results of M. rhesus-protection tests compared with history of recent illness and complement-fixation and mouse-protection tests.

M. rhesus-protection test	History of recent illness			Complement-fixation test			Mouse-protection test		
	Positive	Negative	Total	Positive	Negative	Total	Positive	Negative	Total
Positive.....	13	1	14	9	5	14	14	0	14
Negative.....	11	4	15	6	9	15	5	6	11
Total.....	24	5	29	15	14	29	19	6	25

The percentage of positive complement-fixation results in Cambucy was unexpectedly low in comparison with that in Magé. While this may be due to variation in antigens used, the definite association of complement-fixation results with history of recent illness and the variation shown in the per cent of positives at different periods following suspicious attacks, suggests that the factor of time alone may well account for the difference between the post-epidemic results in Magé and in Cambucy. In Magé, specimens for complement-fixation tests were collected three and four months after the peak of the epidemic, and the percentage of positive results (42.0 ± 2.1) was more than twice that (19.9 ± 1.3) found in specimens taken during and immediately after the epidemic in Cambucy. That a higher percentage might have been expected some weeks later in Cambucy is indicated by the high percentage (55.6 ± 5.0) of positive results in cases with history of attack from six to ten weeks previously.

No significant difference was found between the percentage of immunes observed in Magé with the monkey-protection test (55.3 ± 3.8) and the percentages found in Cambucy with the monkey-protection (48.3 ± 6.2) and mouse-protection (59.3 ± 1.4) tests.

Both Magé and Cambucy failed to show significant differences in the percentage of positive complement-fixation results for the age

groups above and below twenty years, but they did show significant differences in the results of protection tests for these groups. Hughes and Sawyer (5) have recently demonstrated that physiological maturation phenomena are not responsible for the increased immunity observed in the older age groups. Hence, if yellow fever was actually absent from Cambucy during two decades previous to the present outbreak, the residual immunity in the older age groups may be roughly calculated from the formula,

$$x + r\,(100 - x) = y,$$

where r is attack rate in group 19 years of age and under

and y is the percentage of immunes in group 20 years of age and over.

$$x + .515\,(100 - x) = 65.3.$$

Therefore, it is estimated that approximately 28.5 per cent of the present population over twenty years of age has immunity which was acquired prior to the present outbreak. This compares with 41 per cent calculated for Magé by the same formula, using monkey-protection results. If due allowance is made for this preexisting immunity in Cambucy, and their respective immunity rates are applied to the groups above and below twenty years of age, the calculated number of persons recently immunized in the total population of Cambucy is 363. This is an increase of 60 per cent over the number of persons giving history of recent illness (277).

Although Cambucy must surely have been visited repeatedly by yellow fever prior to 1908, only six persons reported having had the disease before the 1930–31 outbreak. Including the cases in the present outbreak, then, there are but nineteen clinically recognized cases in the community on which to base an immunity of approximately 60 per cent in a population of more than 800.

At the beginning of the present study in February, 1931, the mild grippe-like epidemic which had been observed by local clinicians since October, 1930, could not be definitely identified as yellow fever. In fact, a similar wide-spread illness noted by local physicians in Magé prior to the discovery of yellow fever in that town in 1929 (1) was not studied because it did not measure up to the criteria on which diagnosis of yellow fever is usually based.* Had the results of complement-fixation and monkey-protection tests made on sera from Magé not

* In Magé the illness was thought to be malaria, an opinion later shown to be erroneous. In Cambucy, where malaria is not endemic, the febrile outbreak was believed to be grippe.

indicated a much higher degree of population immunity than could be expected on the basis of known yellow fever, this outbreak of "grippe without respiratory symptoms" in Cambucy would have received scant attention. Although yellow fever was known to have been present since November, the number of classical cases was so small that it was hard to believe that these were not sporadic cases occurring in a population largely immunized by previous attack.

The high degree of association found between history of recent illness and positive mouse-protection and complement-fixation results, and the striking variation of the complement-fixation results at different periods after indicated dates of recent illness, may be interpreted as strong evidence that the wide-spread immunity observed in Cambucy was for the most part acquired during the six-months' period from October, 1930, to March, 1931.

The distribution of history of illness by months and blocks shows a remarkable relationship to the distribution of known cases of yellow fever and to the distribution of immunity to yellow fever as shown by the mouse-protection test; and one cannot escape the conviction that the Cambucy epidemic of yellow fever of 1930–31 was composed of several hundred mild infections and less than a score of clinically recognized cases. An attempt to attribute all immunity in the age group below twenty years to the observed epidemic, meets a discrepancy between history of recent illness (28.6 $\pm$ 1.5 per cent) and results of mouse-protection tests (51.5 $\pm$ 2.2 per cent). It would seem then that this outbreak of yellow fever in Cambucy either was not the first since 1908, or was characterized by a large number of truly subclinical infections, which either produced no symptoms or symptoms so mild as to be completely overlooked by the families concerned. Considering available information, the latter hypothesis appears more acceptable than the first.

This large percentage of mild and even subclinical infections is very interesting in the light of the racial distribution (table 6) of the population of Cambucy. No significant difference was found among the percentages of different racial groups reporting history of recent illness; neither was any difference found in the percentages of men and women reporting recent illness, although statistics of yellow fever outbreaks generally show an appreciably higher number of cases among men than among women.

Although the total number of persons in Cambucy shown to be immune to yellow fever greatly exceeded the number giving a history of recent illness, there was found to be a marked correlation between a

high percentage of immunes and a high percentage of persons with history of recent illness. For example, two areas, A and B (table 12), may be outlined in which the percentage of persons found to be immune to yellow fever by the mouse protection test is very high (90.4 ± 1.6) in comparison with the percentage of immune persons outside these areas (47.0 ± 1.7). The percentage of persons in these areas giving a history of recent illness (46.7 ± 2.2), although much lower than the percentage of immunes, is much higher than the percentage of persons outside the areas with history of recent illness. It should be noted that the distribution of immunity in Cambucy is far from uniform; the percentage of immunes varies greatly in different parts of the town. Caution must be observed in drawing general conclusions regarding the percentage of immunes in a given population from the results of immunity tests on a small sample of the population.

Summary and conclusions.

An epidemiological survey of yellow fever was made in Cambucy during and after a small epidemic in this previously endemic area, which had been considered free of the disease during two decades. This survey, based on history of illness and complement-fixation and mouse-protection tests, if interpreted in the light of past history of yellow fever in the area, indicates that the distribution of immunity in Cambucy bore very little relationship to the recognition of classical cases of the disease, but was highly correlated with the distribution of recent cases of so-called "grippe without respiratory symptoms." This epidemic of grippe was characterized by fever, headache, and body pains, and to a lesser extent by nausea, vomiting, and icterus.

A considerable number of persons, on the other hand, even in the younger age groups, were found to be immune to yellow fever, without history of previous illness, indicating that immunization may often occur without illness sufficiently severe to register in the memory of the individual or his family.

The observation made at Magé and Sto. Aleixé (1) that the acquisition of immunity to yellow fever by the native population of a previously endemic region from which the disease has been apparently absent for twenty years may be accompanied by very few classical cases, was confirmed in Cambucy.

The results of control mouse-protection tests on sera from two non-infectible areas are presented, confirming the freedom of this test from any appreciable percentage of falsely positive results.

This survey has thrown further light on the period at which com-

plement-fixing bodies are most commonly demonstrable after an attack. A significantly higher percentage of positive results was obtained during the period from six to ten weeks after the attack than either before or after this time. Second complement-fixation tests performed some eight months after first tests showed great reduction in complement-fixing bodies, but nevertheless the results of these two tests were highly associated.

Certain evidence is presented suggesting that the mouse-protection test may be a more sensitive indicator of immunity to yellow fever than the monkey-protection test. Results of mouse-protection tests, results of complement-fixation tests, and history of recent illness were found to be closely associated.

The distribution of immunity to yellow fever may be far from uniform even in small communities.

The results of the present study indicate that epidemiological investigations, unsupported by laboratory tests and autopsies, are bound to be falsely comforting in endemic yellow fever areas in which there is little movement of foreigners.

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